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STRUCTURAL AND HISTOCHEMICAL CHANGES IN THE LIVER OF FEMALE RATS UNDER THE EFFECT OF INJECTABLE CONTRACEPTIVE

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ABSTRACT

Background: Safe and effective method for contraception is still under continuous search. Injectable contraception is an attractive method and considers to be a suitable alternative for oral tablets. The use of the Medroxyprogesterone acetate (MPA) is a promising way for contraception due to its long mode of action and of minimal risk. So this study was planned to evaluate the structural and histochemical changes induced by (MPA) in the liver of adult female rats as well as testing the degree of reversibility of the changes that may develop after the arrest of its use.

Material and methods: Thirty adult female Albino Rats were used and divided into 3 groups: The first group was considered as a control group. The second group was injected intramuscularly with MPA 4 times with a dose of 2.7 mg/rat every three oestrus cycle. Liver samples were taken one day after the arrest of the last injection. The third group was treated as group two but liver samples were taken 30 days after the arrest of the last injection. Indian ink injection was done in the abdominal aorta for studying the distribution of the blood vessels in the liver. Paraffin sections were prepared and stained with Heamatoxylin and eosin and by PAS stain for structural changes and evaluating the amount of glycogen contents. Frozen sections were prepared for histochemical staining of both acid phosphatase and succinic dehydrogenase enzymes.

Results: The obtained results showed that the use of MPA causes marked vascular congestion in the hepatic vessels and it was of statistically significant when compared with that of the control. Such change was greatly improved after 30 days of the arrest of the injection.

Cellular hepatic changes were in the form of cellular hypertrophy and pale cytoplasmic staining. The cellular arrangement in the liver lobules was not altered.

Changes in the glycogen content showed marked increase in the content of its deposition and after the arrest of the injection it returns back to its normal values. Histochemically it was observed no changes in the enzymatic contents in the liver cells for both acid phosphatase or succinic dehydrogenase enzymes especially when compared with the control date.

Conclusions: It was concluded from this study that there were reversible structural and histochemical changes in the rat liver under the use of the MPA, So it is concluded that the use of MPA could considered as a safe contraceptive method. This study advise the continuous check up on the liver function during the use of such method.

Key words: MPA – Contraception - Liver – Histochemistry- Injection.

INTRODUCTION

Many studies have been performed for finding a suitable contraceptive.

The aim of such studies was to avoid the estrogen content in the oral pills. Long acting injectable progesterone was the suitable choice as an alternative for the oral pills. Two types of progesterone, the first was medroxyprogesterone acetate **(MPA)** while the second was noreth-indrone enanthate **(NET – EN)**. Upjohn pharmaceutical developed MPA under the trade name **(Depoprovera)** and Schering developed (NET – EN) under the name of **(Noristrate)**. MPA was first

used for the treatment of habitual abortion and endometriosis **(Kennedy,1978).** The same drug was used also in the treatment of premature labor **(Coutinho and Souza,1966)** and on that bases small doses of MPA have been used as long acting contraceptive. MPA was prepared in microcrystalline form which delays the absorption after its injection **(Duax et al., 1978).**

Within one week after the injection of 150 mg of DMPA ,its level reaches a peak ranging from 1 to 6.6 ng/ml/serum **(Fotherby et al.,1982).**
These levels remain elevated for 2 to 3 months and then declined steadily.

Elder et al.1984, reported that the extent of conjugation of MPA in the liver is not known, while metabolic clearance studies indicated that MPA is rapidly removed from the blood stream. The rate of metabolic degradation of MPA on its efficacy, it was found that women in different population groups may metabolize an injectable progestin at substantially different rates **(Liskin and Quillin ,1983).**

Matute and Kalkhoff (1972) studied the hepatic gluconeogenesis and glycogen formation in female rats following parenteral administration of sex hormones on the liver carbohydrate metabolism, their results suggested that sex hormones suppress hepatic gluconeogenesis while promoting liver glycogen deposition.

Edress et al., (1991) studied the histopatholical effect of hormonal contraceptive on the liver, kidney and genital organs of female albino rats. They found that the liver was congested and showed portal and interstitial aggregations of lymphocytes and macrophages besides hyperplastic bile ducts.

Attia et al., (1994) , treated female rats with injection of combination of estrogen and progesterone. They noticed focal changes in some hepatic lobules, dilatation of the blood vessels and slight fibrosis in the portal areas 7 days of the injection.

Selman et al., (1995) ,studied the effect of MPA on some organs of the dog. They confirmed the previous findings in the liver and termed the changes as steroid induced hepatopathy.

Amatayakul (1979) showed that the use of MPA does.'t interfere with either enzymatic or most excretory functions.

The aim of the present work was designed to evaluate the hepatic changes under the effect of injectable contraceptive and testing the degree of reversibility of the changes that may be developed.

MATERIAL AND METHODS

Material:

A-Animals: Thirty adult female albino rats of local strain with body weight ranging between (120-140 gm) were used in the current work. Rats were divided into three equal groups:

Group I (Control group), the animals were injected with same dose of the vehicle used in this work.

Group II (Treated group) ,the animals were injected with the drug (MPA) in a dose of 2.7 mg/rat every 3 oestrus cycle for 4 times. Liver samples in this group were obtained one day after the arrest of the injection.

Group III (Recovery group), Such group was designed for testing the degree of reversibility of the possible changes after the injection.

The rats in this group were injected with the same manner as group 2 but liver samples were obtained 30 days after the arrest of the last injection.

B-Drugs and chemicals:

The drug medroxyprogesterone acetate (MPA) is available in aqueous suspension as commercial compound named (Depoprovera) Upghon,USA.

The drug was injected intramuscular in a dose of 2.7 mg dissolved in 0.5ml saline,every 3 oesrus cycle for four injections.The calculate dose were considered according to **Paget and Barnes (1964).**

Methods:

The rats in the different groups were injected with India ink in the abdominal aorta for demonstration of the vascular pattern of the hepatic vasculature. Casting technique was used for calculating the % of

area of filling by the hepatic blood vessels. The samples of liver were obtained and processed for preparation of **paraffin sections**. Two types of stains were used, Hematoxylin & Eosin (Hx&E) for morphological changes and PAS technique for demonstration of the glycogen content in the liver cells. **(clayden,1971).**

Frozen sections were also prepared for histochemical localization for the activity of both acid phosphates and succinic dehydrogenase enzymes. **(Gomori, 1941) , (Pearse ,1975)**

Image analysis system was used for determination of the optical density for glycogen contents in the liver cells and for the enzymatic activity of both acid phosphatase and succinic dehydrogenase enzymes.

The obtained data were analyzed using student (t) test, significant differences between the means of control and treated groups were considered at $p<0.05$ (***Sokal*** and ***Rohif, 1981***)

RESULTS

Morphological changes:

(Figs. 1 – 8 – 9 – 10)

Study of the distribution of hepatic blood vessels was done after injection of India ink through the abdominal aorta. It was noticed that the injection of MPA leads to marked vascular congestion in the hepatic vessels and dilatation in the blood sinusoids . Such congestion wasreturned back to its normal distribution 30 days of arrest of the injection.

2- Changes in the cellular pattern of the liver: (Figs. 5 – 6 – 7)

In this study, it was noticed that control liver showed normal hepatocytic arrangement with central location of the central vein. The hepatic cell cords were separated by the hepatic sinusoids which are arranged in radial manner. In the injected group the liver cells showed hypertrophic changes but not lost their cellular normal arrangement. After 30 days of arrest of the injection with MPA the cells returned back to its normal size.

Histochemical changes:

1- Changes in the Glycogen contents in the liver cells:

(Figs. 2 – 11 – 12 – 13)

After statistical analysis there was statistical significant increase in the contents of glycogen in the hepatocytes of injected group when compared to its contents in the control group. Thirty days after arrest of the injection with MPA there was normal glycogen content in the hepatocytes in the third group which near to its contents in the control group.

2- Changes in the activity of acid phosphatase enzyme:

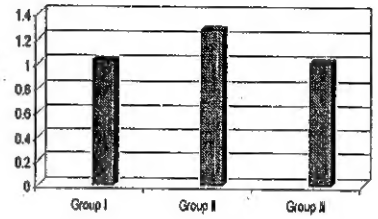
(Figs. 3 – 14 – 15 – 16)

3- Changes in the activity of acid phosphatase enzyme

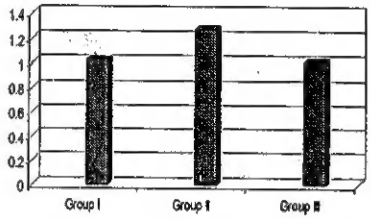
(Figs. 4 – 17 – 18 – 19)

In this study Histochemical changes of both enzymes showed no changes in their activities in the hepatic structure in the different groups.

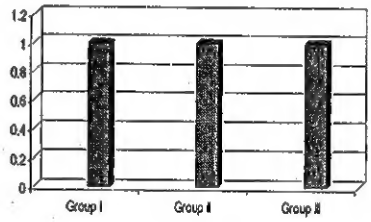
(Fig. 1) Changes in the distribution of the hepatic blood vessels in the different groups of the study under the effect of injectable contracetive



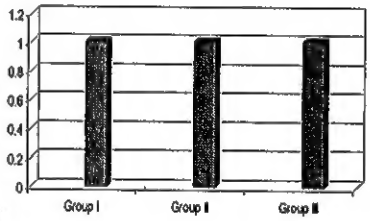
(Fig. 2) Changes in the Glycogen contents in the liver cells in the different groups of the study under the effect of injectable contracetive

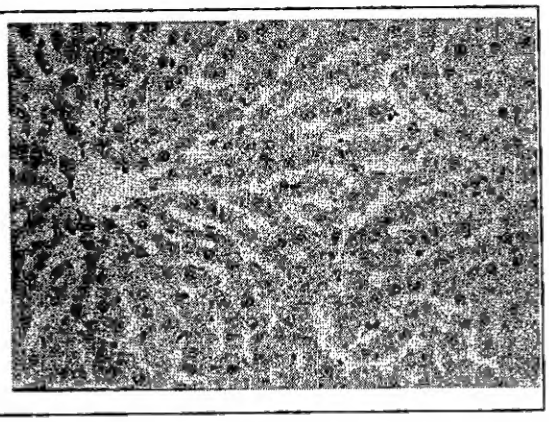


(Fig. 3) Changes in the activity of acid phosphatase enzyme in the liver cells in the different groups of the study under the effect of injectable contracetive

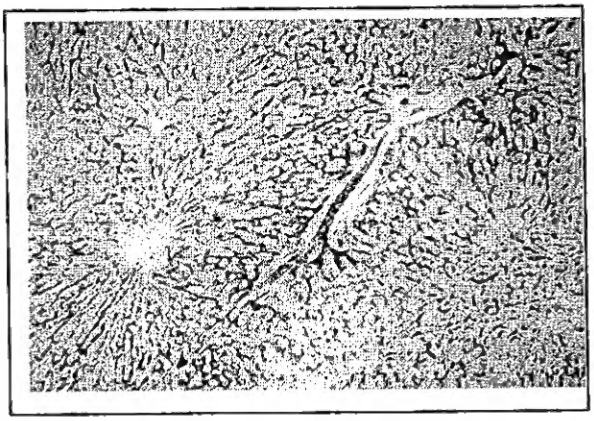


(Fig. 4) Changes in the activity of succinic dehydrogenase enzyme in the liver cells in the different groups of the study under the effect of injectable

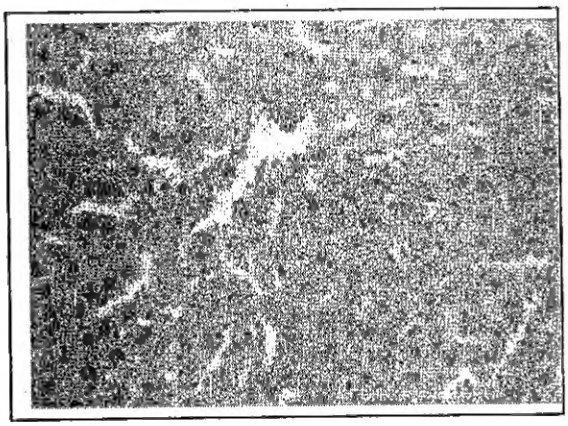




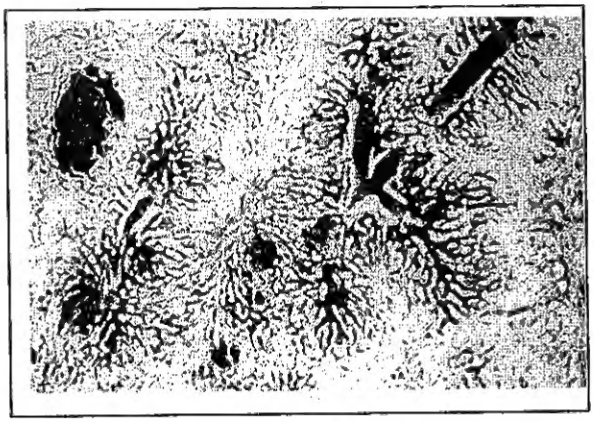
(Fig. 5) Photomicrograph of a section in the rat control liver showing the normal liver cells as well as normal vascular pattern (Hx.E stain X 500).



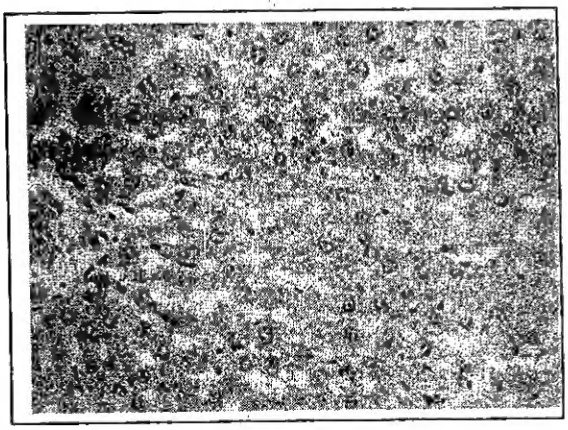
(Fig. 8) Photomicrograph of injected control rat liver showing normal vascular pattern. (India ink injection X 125).



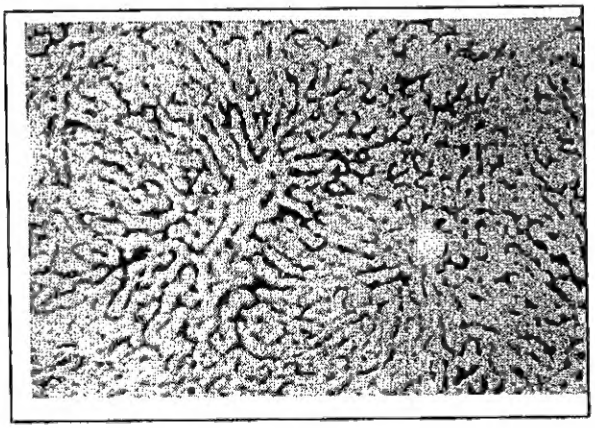
(Fig. 6) Photomicrograph of a section in the rat liver after MPA injection showing the swollen l iver cells . (Hx.E stain X 500).



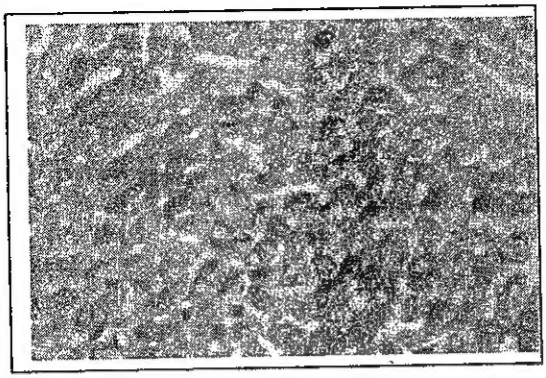
(Fig. 9) Photomicrograph of injected rat liver after MPA injection showing marked vascular congestion. (India ink injection X 125).



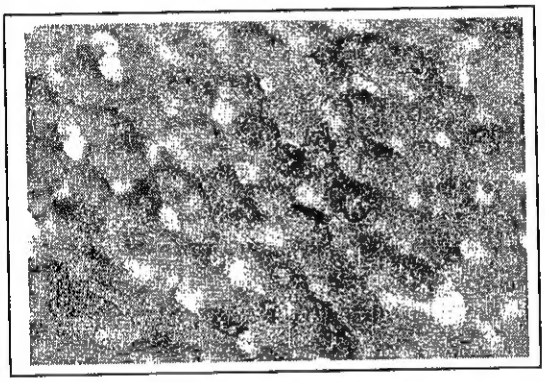
(Fig. 7) Photomicrograph of a section in the rat liver 30 days after the arrest of injection showing the normal liver cells as well as normal vascular pattern . (Hx.E stain X 500).



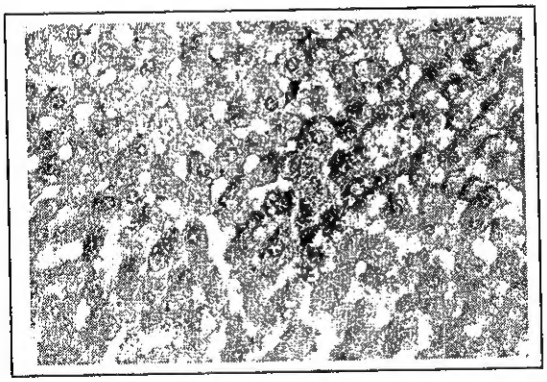
(Fig. 10) Photomicrograph of a section in the rat liver 30 days after the arrest of injection showing normal vascular pattern . (India ink injection X 125).



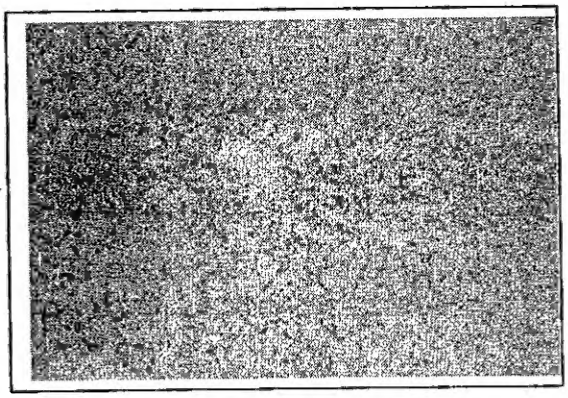
(Fig. 11) Photomicrograph of a section in the control rat liver showing the normal distribution of Glycogen contents. (PAS stain X 250).



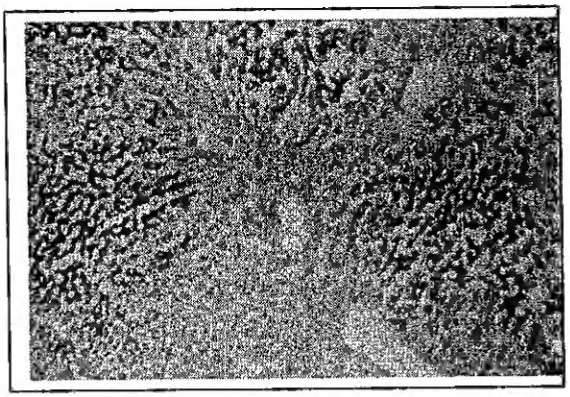
(Fig. 12) Photomicrograph of a section in the rat liver after MPA injection showing marked increase in the Glycogen contents. (PAS stain X 500).



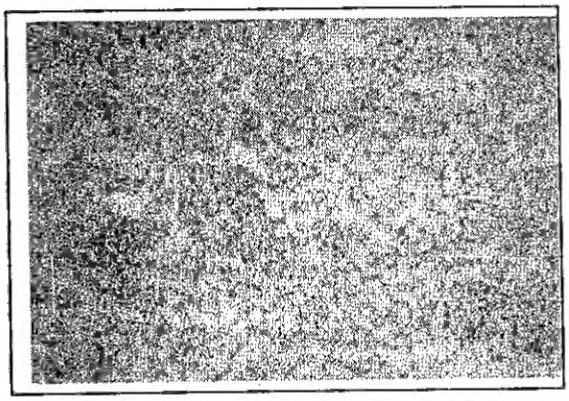
(Fig. 13) Photmicrograph of a section in the rat liver 30 days of arrest of MPA injection shwing the return of the noemal Glycogen contents .(PAS stain . X 250).



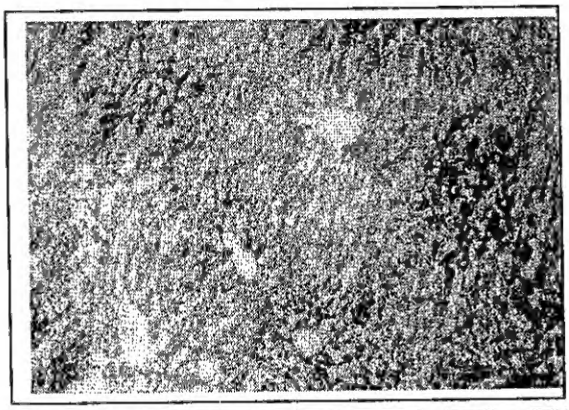
(Fig. 14) Photomicrograph of frozen control section in the rat liver showing the distribution of normal acid phosphatase enzyme reaction.
(Gomori stain X 125)



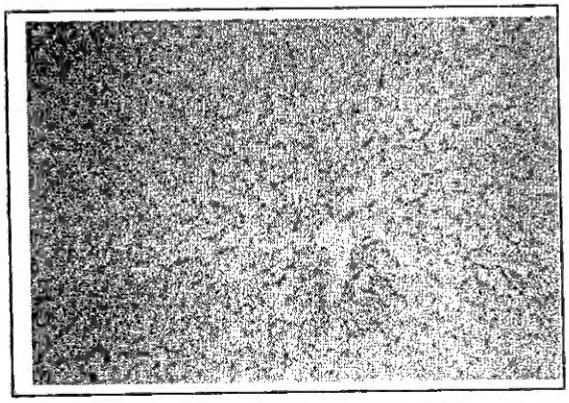
(Fig. 17) Photomicrograph of frozen control section in the rat liver showing the distribution of normal succinic dehydrogenase enzyme reaction.
(Nachlas stain X 125).



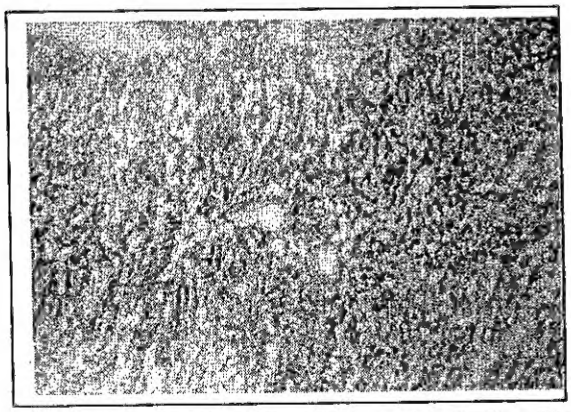
(Fig. 15) Photomicrograph of frozen section in the rat liver injected with MPA, showing no changes in the activity of acid phosphatase enzyme
(Gomori stain X 125). .



(Fig. 18) Photomicrograph of frozen section in the rat liver injected with MPA, showing no changes in the activity of succinic dehydrogenase enzyme .
(Nachlas stain X 125).



(Fig. 16) Photomicrograph of a frozen section in the rat liver 30 days of arrest of MPA injection , showing no changes in the activity of acid phosphatase enzyme
(Gomori stain X 125).



(Fig. 19) Photomicrograph of a frozen section in the rat liver 30 days of arrest of MPA injection , showing no changes in the activity of succinic dehydrogenase enzyme (Nachlas stain X 125).

DISCUSSION

The diabetic patients need alternative therapies to control all the pathological aspects of diabetes and the high cost and poor availability of current therapies in developing countries **(Marles and Farnsworth, 1995).**

The traditional antidiabetic plants might provide this useful source of new oral hypoglycemic compounds. So, this study is a step to evaluate and follow up the effect of some water extract of medicinal plants as a hypoglycemic agent.

The present results, revealed loss in body weight gain in diabetic rats when compared with the control rats. This loss may be explained by inhibition of synthesis of DNA and RNA in the diabetic animals or it is attributed to different side effects on the ability to use carbohydrates including lipolysis, glycogenolysis and acidosis. This result is mainly due to destructions of β-cells which lead to sudden drops of insulin secretion **(Abdel-Moneim *et al.*, 1999a; Ganong, 2003; Helal *et al.*,2003 and Rawi *et al.*, 1996).**

Our data also, showed an increase in the body weight gain in all treated groups when compared with diabetic one. The mixture may stimulate most aspects of carbohydrate metabolism, including rapid uptake of glucose by the cells, enhanced glycolysis, enhanced gluconeogenesis, increased rate of absorption from the gastrointestinal tract and even increased insulin secretion with its resultant secondary effects on carbohydrate metabolism (*Guyton* and *Hall, 2000*). Ferula assa-foetida, Boswellia carterii Birdw and Commiphora myrrha treated groups showed an increase in the body weight gain when compared with control group . This increase in the body weight gain may be due to the pervious suggestion in addition to the activities of these plants in strengthening the gastrointestinal tract by increasing both the rate of secretion of the digestive juices and the motility of the gastrointestinal tract (*Guyton,* and *Hall, 2000*), so they are taken for indigestion (*Chevallier, 1996* and *Duke, 2002*) .

While, Nigella sativa treated group showed a decrease in the body weight gain when compared with control group. This probably rise from its claim to result in nutrient partitioning so that ingested calories will be directed to muscle, rather than fat and/or attempt to physically affect gastric satiety by filling the stomach (*Heber, 2003*). And possibly spring from its anti water retention action **(*Duke, 2002*).** Or may be attributed to increase plasma leptin concentration which travels to the appetite control center in the brain and tells it to " stop eating " **(*Ebihara et al., 2001*).**

Severe hyperglycemia in diabetic rats recorded in the present work can be considered as a direct reflex to the marked hypoinsulinemia caused by the selective destructive cytotoxic effect of alloxan on the β-cells of the pancreas which has a direct effect on their membrane permeability by causing failure of ionic pumps and increased cells size. It also inhibits intracellular energy generation, insulin secretion and causes sudden activation of quiescent cells for a high level of protein synthesis and produced rapid and massive beta cell death which leading to a decrement in ß-cells number **(Majno and Joris, 1999).**

The destructive effect of alloxan on ß-cells may be also attributed to its ability to inhibit enzymes of the tricarboxylic acid cycle and Ca^{2+} dependent dehydrogenases in β-cell mitochondria, causing ATP deficiency, cessation of insulin production and cell necrosis **(Shafrir, 2003).**

The results of the present study also showed β-cells with vacuolated cytoplasm in the diabetic group. Vacuolation of the islet is the most prominent lesion associated with functional islet abnormality and development of hyperglycemia **(Bolaffi *et al.*, 1986 and Kessler *et al.*,1999).** Also, the vacuolation may be due to the diabetogenic action of alloxan which induced highly reactive oxygen radicals, which are cytotoxic to β-cells **(Fischer and Homburger, 1980).**

According to **Yamamoto *et al.* (1981)** and **Ronald (1988)** the fragmentation of nuclear DNA of pancreatic β-cells seems to be important for the development of diabetes and supposed to be resulted from the accumulation of superoxide or hydroxyl radicals in the β-cells.

Marles *et al.* (1995) suggested that, the hypoglycemic effect of some medicinal plants could be attributed to factors other than stimulation of insulin release only, e.g. their effect on the number and /or affinity of insulin receptors on target cells and the post-receptors of these cells.

Abdel Moneim *et al.* (1999a) reported that the hypoglycemic effect of *Nigella sativa* may be attributed to an increase in the islet numbers and to its effect on the time-course of glucose resorption from the intestine.

On the other hand, the *Nigella sativa* treated group showed insignificant change in beta cells number and diameter as compared to the normal group. This plant may have a stimulatory effect on the division of β-cells or may contain non-metabolizable 2-deoxy and 3-O-methylglucoses, which share the entry site block the diabetogenic action of alloxan and restore insulin production **(Shafrir, 2003)**.

The significant hypoglycemic and insulinotropic effect induced in diabetic rats by such plants treatment may result from its effect on the time course of glucose resorption from the intestine. It also has an effect on peripheral tissues and regeneration of islet of Langerhans leading to lower blood glucose level. The treatment with *Nigella sativa* induced islet cells regeneration with increased number of β-cells **(Abdel-Moneim *et al.*, 1999 and EL-Daly,1994)**.

On the other hand, all treated groups recorded insignificant change in beta cells number and diameter as compared to normal group. These plants may have stimulatory effect on the division of beta cells to divided and /or contain nonm-etabolizable 2-deoxy and 3-O-methylgl-ucoses,which share the entry site block The diabetogenic action of alloxan and restore insulin production (***Shafrir,2003***). ***Augusti*** and ***Sheela*** (***1996***) mentioned that some plants exert their effect on beta cells through both protection of the already present beta cells due to their antioxidant effect and through stimulation of the beta cells to release insulin. In the present study it seems that the treated plants exert their action on the diabetic pancreas or due to close connection of admixture of islet cells, acinar cells and ductal epithelium which may resulted metaplastic change of acinar cells and ductal cells into islet cells under unknown stimulus (***Hisoha*** and ***Horie, 1990***).

In the present investigation, the treatment of diabetic rats with mixture and Boswellia carterii Birdw caused a significant hypoglycemic effect with a significant increase in serum insulin level and regenerated B-cells . In agreement with these findings ***Al-Awadi et al. (1988)*** reported that the mixture of (Nigella sativa, Aloe vera, Commiphora myrrha, Gum albunium and Ferula assa-foetida) is a potent hypoglycemic agent . The antidiabetic action of these plants extract may, at least partly, be mediated through decreased hepatic gluconeogenesis ***(Al-Awadi et al., 1991)*** . The decreasing of blood glucose level by the mixture and Boswellia carteii Birdw is secondary to enhanced insulin secretion, decreased insulin resistance and glycogen synthesis activation. it seems to have a direct action on insulin secretion through stimulation of secretion of the Golgi complex ***(Bever*** and ***Zahand, 1979)*** or it may stimulate insulin secretion through the B-cell receptor and possibly through a direct effect on intracellular calcium transport (***Campbell et al., 1991)*** .

Conclusions: The water extract of the mixture and each of the plants used appeared to be useful agents in reducing the hyperglycemia by increasing insulin level and regenerating beta cells of the pancreas. More studies on these plants must be done with different doses and for different periods before recommending their use.

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